

Effects of Certain Mineral Elements
on Cercospora Leafspot of Peanuts

By

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Abstract of Dissertation Presented to the Graduate Council
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EFFECTS OF CERTAIN MINERAL ELEMENTS
ON CERCOSPORA LEAFSPOT OF PEANUTS

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Two varieties of peanut (Arachis hypogaea L.), 'Early Runner' and 'Florigiant', were grown for 55 days in various nutritional regimes, using modified Hoagland's solutions, during May to August in the greenhouse with temperature and relative humidity varying from 22-35° C and 40-96 per cent respectively. Typical deficiency symptoms for the nutrients calcium, magnesium, potassium, and phosphorus developed in both varieties. However, phosphorus-deficient plants of 'Early Runner' variety developed split stems longitudinally along their axes up to the node of the last intact leaf.

Levels of the 4 elements were significantly different in the leaves of both varieties for all nutritional treatments except for the potassium level in 'Florigiant'. The treatments significantly varied iron concentrations in both varieties but did not significantly affect the nitrogen

contents in either variety. The increase of each element separately in the nutrient solutions produced an increase of that element in the leaf tissues. The interaction of ions in the complete solutions interfered with their absorption by the plants.

Nutrient levels in the leaves of both varieties were not similar and neither did both varieties respond alike in susceptibility to the pathogen, Cercospora arachidicola Hori. Disease incidence was greatest in variety 'Early Runner' on plants grown in phosphorus-deficient solution but it was not significantly different from that of plants grown in magnesium-deficient, potassium-deficient, magnesium-2.0, potassium-0.5, and calcium-0.5 solutions. 'Florigiant' plants did not show any significant differences in susceptibility, and the highest disease incidence was on plants grown in potassium-2.0 solution. No direct relationship between disease percentage and percentage defoliation was observed.

INTRODUCTION

Cercospora leafspot is generally regarded as a very destructive fungal disease of peanuts, Arachis hypogaea L., and occurs wherever peanuts are grown. It causes premature leaf shedding and is responsible for about 30 per cent reduction in yield of hay in the United States (35). The productive capacity of the plants can be so reduced that the economic losses are estimated to be from 15 to 50 per cent of the yield in many areas of the world (24).

The species Cercospora arachidicola Hori and Cercospora personata (Berk. and Curt.) Ellis and Everhart were established by Woodroof (50) and are the two most commonly associated with the leafspot disease. However, a third species Cercospora canescens Ellis and Martin has been reported on peanuts in Northern Nigeria (36) and Jamaica (30). The ascigerous stages of C. arachidicola and C. personata were described by Jenkins (25), as Mycosphaerella arachidicola Jenkins and Mycosphaerella berkeleyi Jenkins, respectively. Nevertheless, the names associated with the conidial stages are still widely used in the literature. The common names 'early' and 'late' leafspot have been applied to the two diseases because in the United States C. arachidicola is usually found earlier in the growing season and is the

predominant species, whereas C. personata is found later and is less abundant. Miller (35) made 697 isolations from diseased leaves obtained from ten Southern States: 692 of them were C. arachidicola; the remaining 5 were C. personata.

No outstanding progress has been made incorporating resistance in any of the cultivated varieties. Breeding of peanut varieties resistant to *Cercospora* leafspot is further complicated in that resistance to each C. arachidicola and C. personata is independently inherited (1, 21). Chemicals have been used effectively and profitably against the two diseases. Sulfur is the most widely used chemical and the addition of a copper compound to it increases fungicidal effectiveness.

Based on a review of the literature on the biology of C. arachidicola and C. personata, Miller (34) concluded that application of fertilizer to peanuts had no apparent effect on the leafspot disease. However, the relative severity of the disease was changed whenever nitrogen, potassium, manganese, or calcium was present in limiting or excessive amounts for good peanut production. Bledsoe et al. (2) reported in 1949 that peanut plants grown in magnesium-deficient cultures were more susceptible to leafspot.

It is generally accepted that the susceptibility of a plant to disease is related to the nutritional condition of the host. The study reported here was designed to investigate the effect of different levels of 4 nutritional elements, namely, calcium, magnesium, potassium, and

phosphorus on the severity of Cercospora leafspot on peanut
caused by C. arachidicola.

LITERATURE REVIEW

Pathogen and Disease

Woodroof's capacious study of 1933 (50) aided greatly in clarifying the confusion in the description and nomenclature of the two *Cercospora* attacking the leaves of peanut. *Cercospora arachidicola* Hori was first described in Japan in 1919 and *C. personata* (Berk. and Curt.) Ellis and Everhart in 1875 by Berkley as *Cladosporium personatum* which was renamed by Ellis and Everhart 10 years later.

Jenkins (25) studied the life history and cytology of the two peanut leafspot fungi. Each fungus was found to produce spermagonia and perithecia in addition to conidia. He proposed *Mycosphaerella arachidicola* sp. nov. for the perithecial stage of *C. arachidicola* and *M. berkeleyii* sp. nov. for the perithecial stage of *C. personata*. The development of spermagonia and perithecia of both fungi is initiated during early fall. He stated that the perfect stages play an important part in the survival of the fungus and in establishing primary infection in North America. There are no reports of the perfect stages being found in other peanut growing areas of the world.

The morphological characteristics of *C. arachidicola* were described in detail by Jenkins (25), Chupp (9), and

Woodroof (50). The conidia are colorless to pale olivaceous, obclavate, measure $37.8-108 \times 2.7-5.4 \mu$, and are 3-12 septate. Their length and septations are influenced by weather conditions at the time of production. They are borne on yellowish brown, continuous or 1-2 septate conidiophores which emerge from a stroma in clusters. Similar characteristics of C. personata which are quite distinct from C. arachidicola were also described (9, 25, 50). The conidia are light to dark olivaceous brown, cylindrical or obclavate, measure $18-70 \times 5-11 \mu$, and are 1-8 septate. The reddish brown, continuous or 1-3 septate conidiophores develop in distinct concentric tufts from heavy stromatic bases.

According to Jenkins (25) germination of conidia occurred within 3-8 hours when moisture, oxygen and temperature conditions were favorable. Jenkins (25) and Woodroof (50) observed that germ-tubes were not limited to the terminal cells of the conidia but developed from any of several cells.

Inoculation tests with conidia of C. arachidicola carried out by Jenkins (25) indicated that the germ-tubes may enter either surface of a leaflet through lateral faces of epidermal cells or through open stomata. However, Hemingway (20) and Abdou (1) reported that germ-tube penetration occurred only through stomata. They also established that there is a critical stomatal size above which the stomata are penetrable. Hemingway observed that no penetration occurred in stomata less than 13.4μ in length and that the

majority of stomata penetrated were over 16.7 μ long. Such findings were also supported by Gibbons and Bailey (16) and D'Cruz and Upadhyaya (11). Working with Cercospora beticola Sacc. and sugar beet (Beta vulgaris L.), Pool and McKay (42) found a close relationship between size of stomatal pores and hyphal penetration. Below a stomatal pore size of 15 μ no penetration was observed. Abdou (1) further suggested that there was a substance passing from open stomata which acted as an attractant to the growing germ-tube during incubation.

After an incubation of 6-7 days from infection, a pin-head lesion was the first visible symptom on the upper leaf surface (19). From the lower surface, small areas were observed in which the epidermal cells had collapsed and lost connection with the underlying mesophyll (25). As the spots became older, Jenkins (25) claimed that the two diseases incited by C. arachidicola and C. personata were easily distinguishable.

According to Jenkins (25) the spots associated with C. arachidicola are irregularly circular, reddish brown to dark brown, almost black, measure 1 mm to 1 cm across, and often coalesce. A chlorotic halo which surrounds each lesion eventually becomes bright yellow. On the lower surface of the leaf the lesion is usually a lighter brown color and the halo is less pronounced.

Although the halo has been accepted to be a characteristic feature, Jackson and Bill (24) reported that a halo

was not always present and suggested that it might be a function of a varietal reaction. Jenkins (25) surmised that the halo was perhaps related in some way to the carbohydrate content of the leaf cells since its development was best on leaves with high carbohydrate content. In the case of C. personata the spots are dark brown to black, nearly circular, slightly smaller, 1-6 mm, and the yellow halo is present only on the more mature spots on the upper leaf surface.

With C. arachidicola the intercellular mycelium kills cells in advance of its growth and the hyphae then become intracellular. In contrast, C. personata hyphae remain intercellular but produce botryose haustoria within the host cells (1, 25, 50). Sporulation in both species does become amphigenous but it is at first epiphyllous in C. arachidicola and predominantly hyphophyllous in C. personata with the structures arranged in concentric circles.

A general characteristic of many species of Cercospora is that they do not sporulate in culture and, if so, only sparsely. When common laboratory media were utilized, several investigators (29, 31, 37, 39) had difficulty in producing conidia in large quantities. In the case of C. arachidicola, Woodroof (50) obtained mycelial growth as a dense black mass but no conidia were observed. Jenkins (25) was more successful in achieving sporulation in culture. He used several different agar media, and within 5-7 days at room temperature conidia were produced on whitish, sharply delimited, pulvinate colonies.

Because of the slow-growing nature of the leafspot fungus, procuring an ample supply of inoculum for pathogenicity studies has been a critical problem. In an attempt to find a satisfactory medium for cultural development of inoculum for C. arachidicola, Miller (35) evaluated several synthetic and semi-synthetic media. He found that spore formation was closely related to rate of growth and that the greatest number of spores could be formed when a 2 per cent concentration of glucose was included in his basal agar medium. However, he concluded that a successful method of preparing large quantities of conidia for inoculum was not established.

Landers (29) reported that in a starch-yeast extract medium adjusted to pH 4.5 C. arachidicola grew and sporulated well. The medium was continuously shaken and incubated at 30° C to prevent solidification. After a period of 2 weeks an adequate suspension of conidia for pathogenicity studies was obtained. He later developed a chemically defined medium and found that thiamine was a growth-limiting factor. Working with Cercospora personata in pure culture, Shanta (45) found that thiamine was necessary for subsequent growth and sporulation.

Abdou (1) recognized the necessity to develop a procedure to induce consistently good sporulation of the leafspot fungus in a very short time. He found that peanut leaf extract contains the essential nutrients for sporulation and the effectiveness of this substrate was only equalled by

oatmeal agar. The addition of water extract of 30 grams of peanut leaves to water agar and Difco Lima Bean agar improved their usefulness as satisfactory substrates. The stimulatory effect of host-leaf extract, when added to water agar, on the sporulation of species of Cercospora was determined by Kilpatrick and Johnson (28). Eleven different species of Cercospora were tested. Carrot leaf decoction agar was superior to other substrates for all species. Sporulation of C. brachiata Ellis and Everhart., C. canescens Ellis and Martin, C. penniseti sp. nov., and C. zebrina Passerine was abundant and occurred within 3-5 days.

Factors Affecting Disease

Climatic Factors

There are factors other than substratum that have to be taken into consideration when Cercospora species are cultured for maximal sporulation. In the case of C. arachidicola light was not required but an optimal temperature was desirable (29, 35). Miller (35) found that the temperature range for the fungus is 4-35° C. The optimal temperature for its maximal growth was about 32° C and about 30° C for best sporulation. The growth of all the isolates with which he worked was checked at a temperature of about 37.5° C and their survival was threatened at temperatures from 37.5-40° C. In their study with C. beticola Sacc., Calpouzou and Stallknecht (8) found that an interaction between light and temperature affected sporulation. On sugar beet molasses, sporulation was stimulated by light at 15° C, depressed by light at 30° C, and either response could occur at 22.5° C.

The isolate of C. arachidicola that Abdou (1) used throughout his investigation was highly stable in culture and did not lose either its ability to sporulate or its pathogenicity. Miller (35) found that each isolate remained phenotypically constant under similar environmental conditions, but when those conditions differed so did the phenotypic nature of the culture.

Although the first symptoms of the disease incited by both species of Cercospora could be detected as early as 6-7 days after infection (20), such expression can be suppressed for 3 weeks to a month (25). In hot weather, symptoms did not appear until after 4 weeks. Jensen and Boyle (26) reported that large increases in the amount of disease in field plots were correlated with periods of high relative humidity. Temperatures during the high relative humidity periods were usually in the 20s. During the 2 years of observation, rains occurred before and during the period of rapid increase in infection and it was assumed that adequate inoculum was always present. Their data showed that during the days when disease increased rapidly there were several consecutive periods of more than 10 hours when relative humidity was 95 per cent or higher. In Sturgeon's investigation with C. arachidicola (48) he found that high relative humidity was more conducive to severe infections and such a condition was better provided in a moist chamber than under an overhead mist system. The percentage of infected leaflets and disease severity increased with the length of time the

inoculated plants remained in the moist chamber; 96 hours proved to be sufficient time. Miller (35) observed that infection was greater if a light film of water could be maintained on the leaflets for several days after inoculation. Lyle (32) noticed that potted plants developed leafspot symptoms within 9 days after inoculation. During that period moisture was abundant and the temperature ranged from 22-34° C. Miller (35) reported that the optimal temperature for disease development lies within a few degrees above and below 28° C.

During the months of July to October Sulaiman and Agashe (49) recorded the intensity of the disease, temperature, relative humidity, and rainfall in four locations. They found that certain conditions were more favorable for the development of the disease than others and concluded that the optimum conditions for the disease were an average rainfall of 79.4 mm, a range of temperature of 20.1 -33.1° C, and a relative humidity of 62.7 per cent. Such conditions prevailed during the month of October in Maharashtra State, India.

In a greenhouse study with the *Cercospora* leafspot of banana, Goos and Tschirch (17) reported that the conditions most favorable for disease development were daily exposure to low humidity, accompanied by night misting in a closed chamber, and high soil moisture. The rate of disease development appeared to be related to the moisture relations of the plant. They concluded that although germ-tube development

was more extensive under continuous misting, intermittent high and low humidities were more conducive to stomatal penetration than were constant high humidities or exposure to continuous misting.

In addition to the areas of the foliage that are parasitized and killed by the fungus, further loss of photosynthetic tissue was enhanced when leaflet and leaf fall began. Although the peanut plant continued to produce new leaves, leaf shedding resulted in early death of the plant if it was severely attacked. Gibbons (15) noted that, by causing defoliation, Cercospora hastened the maturity of the plant. Defoliation was such a prominent character in the disease cycle that Gibbons (16) utilized it heavily as a measure of the reaction of C. arachidicola to different species of Arachis.

Miller (35) established that the amount of leaf shedding of peanut could be correlated with the amount of leaf spotting. Although he found that leafspot counts were the simplest means of determining the level of infection, a more accurate estimate of the injury could be obtained by making relative comparisons of leaf defoliation. In his view, leaf shedding was influenced by a product produced by the fungus or the infected plants and such a substance could be retarded by low temperature. He concluded that the larger the lesions, the sooner leaf shedding began if favorable temperature prevailed. Lesions developed slowly if temperatures were below optimum.

Sturgeon (48) found that infection was directly related to defoliation; however, the highest disease ratings were not associated with the greatest defoliation. He also indicated that a substance might be produced in the host-pathogen association which initiated formation of the abscission layer.

The main pathogenic effect of the fungus Omphalia flavida (Cke.) Maubl. and Rangel on coffee is the severe premature defoliation which it causes (44). The explanation offered for the leaf drop is that the plant's growth hormones are inactivated by the organism. Leaf drop occurs much earlier if a lesion is at the base of the leaf or on the petiole. It was proven that an oxidative enzyme produced by O. flavida inactivates the plant's auxin, indole-3-acetic acid. A similar enzyme may be associated with defoliation of peanut as a result of infection by C. arachidicola.

Resistance

The inference that resistance to *Cercospora* leafspot increased with maturity was based on the following observation: the number of days between observed infection and date of leaflet drop increased with plant age (48). Miller's (35) investigation of the influence of plant age on infection did not completely support the above idea. Of the five plant ages he worked with, the oldest plants, 63 days old, had the least percentage of infected leaves, but the 20-, 27-, and 40-day-old plants had less than that of the 34-day-old plants. Lyle (32) did not report in regards to the numbers of lesions but he did state that 38-day-old plants showed symptoms as soon as did 42-, 52-, and 60-day-old plants.

Since some peanut varieties show differences in their reaction to leafspot, Miller (35) considered nutrient differences as one of the factors influencing such behavior. He found no relationship between the amount of thiamine, protein, ether extract, and crude fibre content of the seed of ten peanut varieties and their resistance to C. arachidicola. However, there was a relationship between the riboflavin of the seed content and the disease intensity. Varieties with the highest amount of riboflavin were relatively more resistant. Hemingway (20) correlated some degree of resistance with the growth habits found in the decumbent or runner varieties. The differences Abdou (1) observed in his studies of host-parasite relations indicated that resistance of leafspot fungi probably is governed by quantitative genes and its inheritance to each of the two organisms is independent as suggested previously by Higgins (21).

Nutrition

Cercospora leafspot.--There are very few reports in the literature dealing with the predisposing effects of the nutritional status of the peanut plant to *Cercospora* leafspot. Burkhart and Collins (7) in 1941 noted that gypsum applications conditioned the peanut plant of the 'Virginia Bunch' variety in such a manner that the foliage became very susceptible to pathogenic leafspot injury and subsequent severe defoliation resulted as the plants approached early maturity. In 1946 Miller (34) reported that plant nutrients applied to soil as fertilizer apparently had no effect on

the severity of the leafspot disease. From further observations over a period of 4 years (35), he noticed that whenever nitrogen, potassium, manganese, or calcium was present in limiting or excessive amounts for good peanut production the relative severity of the disease was invariably associated with premature or delayed ripening of the nuts.

In a nutritional study with the variety 'Dixie Runner', Bledsoe et al. (2) found that the plants grown in a magnesium-deficient culture for a period of its growth developed the leafspot disease to an appreciable extent. These plants were 103 days old and magnesium had been withheld for 28 days. Disease symptoms were observed first on the leaves which first showed symptoms of nutritional disturbance, and the progress of the disease followed the same order of development as that of the deficiency, affecting the youngest leaves on the old branches and then the younger leaves on all branches. Shanta (46) found in field trials that nitrogen and phosphorus applied as fertilizers significantly increased the disease incidence and potassium fertilization decreased it to a small extent.

Other leafspots.--In a series of four papers, Bloom et al. (4, 10, 37) and Muse and Couch (38) reported on the influence of environment on diseases of turfgrasses. They worked with the following four diseases: (1) Rhizoctonia brown patch incited by Rhizoctonia solani Kuhn, (2) Sclerotinia dollar spot incited by Sclerotinia homoeocarpa F. T. Bennet, (3) Phythium

blight incited by Phytium ultimum Snow and P. aphanidermatum (Edson) Fitzpatrick, and (4) Corticium red thread incited by Corticium fuciforme (Berk.) Wakef. They are all important foliar diseases of turfgrasses. In each case nutrition was one of the many environmental factors studied in regard to its influence on the development of each disease. Although alterations in disease incidence were observed in each host-pathogen system, they resulted from multiple interactions of nutritional imbalance, host variety, and time of year the plants were grown. A low balanced medium decreased disease severity in the case of Sclerotinia dollar spot and Phytium blight, whereas it increased disease severity with Rhizoctonia brown patch. High or low levels of potassium or phosphorus had no influence on Rhizoctonia brown patch or Sclerotinia dollar spot. Calcium-deficient nutritional regimes produced plants with significantly increased disease susceptibility to Phytium blight and Corticium red thread.

Hobbs and Waters (23) investigated the effect of three different levels of nitrogen and potassium on severity of Botrytis cinerea Pers. ex Fr. infection of Chrysanthemum morifolium (Ramat.) Hemsl. in field plots. They found that disease severity was affected markedly by different nitrogen levels, but potassium had little effect. As the total salts N and K_2O were increased disease severity increased.

Stall et al. (47) also investigated the same pathogen inciting Botrytis gray mold on tomato plants. Their study emphasized the effect of the balance between calcium and

phosphorus in the plant on the incidence of the disease. They found that with high calcium and low phosphorus gray mold decreased, but increased in the presence of low calcium and high phosphorus. High or low amounts of both calcium and phosphorus produced similar disease incidence.

METHODS AND MATERIALS

The cultures of Cercospora arachidicola were isolated from naturally infected Arachis hypogaea varieties growing in experimental plots at Gainesville, Florida. Infected leaflets were washed in running tap water for 1-2 hours. After removing the excess water, they were placed adaxial side up on moist filter paper in large covered petri dishes and incubated at room temperature.

After 72 hours of incubation conidia were easily removed on the tip of a fine glass needle under a binocular dissecting microscope. It was helpful to moisten the tip of the needle with agar medium or sterile water containing a drop of Polyoxyethylene (20) sorbitan monolaurate (Tween 20). The conidia were lightly streaked on the surface of 2 per cent water agar medium and incubated at room temperature. To obtain more discrete single spore colonies, conidia removed from sporulating leaf lesions were suspended in a small volume of sterile water containing a drop of Tween 20, and were spread on the surface of 2 per cent water agar medium with a sterile L-shaped glass rod. After an incubation period of 4-5 days at room temperature, conidia germinated on both sets of plants. By the 7th day, single spore colonies were transferred to peanut leaf agar medium (1)

which was routinely used for all cultural work. The colonies sporulated about a week after incubation at room temperature. Isolates were maintained by transferring them biweekly to fresh peanut leaf agar plates.

About 2 weeks prior to inoculation of the plants, cultures were increased by spreading a conidial suspension on the surface of peanut leaf agar plates. Individual colonies were transferred to fresh agar plates and kept at room temperature until they were used. Since no prominent pathogenic variability occurred among the different isolates in preliminary tests, several isolates were pooled when inoculum was being prepared. A concentration of 2,000-5,000 spores per ml was adequate for disease evaluation.

Inoculum was prepared by removing colonies from several plates and immersing them in 100 ml of sterilized water containing 3 drops of Tween 20. The flask was agitated gently for a few minutes. A spore count of the suspension was determined with a Spencer Bright-line hemacytometer. The spore suspension was adjusted to a concentration of 2,000-5,000 spores per ml by addition of sterilized water plus Tween 20 (2 drops of Tween 20/100 ml water).

When culture methods failed to provide adequate inoculum, naturally infected leaves from the field having 1-4 discrete lesions were used as a source of spores. Leaves were washed in running tap water for 5 hours, rinsed in distilled water, and incubated in large covered petri dishes

for 3-4 days at room temperature. Sporulating lesions were cut from the leaflets and placed in 100 ml of sterilized water containing 3 drops of Tween 20. After agitation with a sterile glass rod, the suspension was decanted and the spore concentration adjusted.

Seeds of 'Early Runner' and 'Florigiant' varieties were grown in sterile vermiculite moistened with distilled water, under a 14-hour light cycle, with temperatures 24-35° C and relative humidities of 45-65 per cent. Eight days after planting, healthy seedlings in the 4-leaf stage were removed and their roots washed in distilled water. Cotyledons were removed with a razor blade which was washed in an aqueous solution of HgCl_2 (1 g/1000 ml) between excisions.

The chemical composition of the 15 nutrient solutions used as nutritional treatments is presented in Table 1. As described by Hoagland and Arnon (22), the chemical composition of each of the 4 deficient solutions (calcium, magnesium, potassium, and phosphorus) differed from that of the complete solution. The constituents of these 5 solutions were modified to obtain 0.5 x and 2.0 x concentrations of each. To obtain the 0.5 x and 2.0 x concentrations of the 4 elemental solutions only the element under investigation was varied while other nutrients remained at the same level. The volume of the supplement and iron were constant in all solutions except 2.0 x complete, where they were doubled. The reaction of each solution was adjusted to approximately pH 6 by adding 0.5 N NaOH or 0.1 N H_2SO_4 .

Table 1. Chemical composition of the 15 nutrient solutions used as nutritional treatments to grow two varieties of peanuts.

Stock solutions	Complete			Ca			Mg			K			P		
	0.5	1.0	2.0	0.0	0.5	2.0	0.0	0.5	2.0	0.0	0.5	2.0	0.0	0.5	2.0
	ml/l of nutrient solution														
M $\text{NH}_4\text{H}_2\text{PO}_4$	0.5	1	2	--	--	--	--	--	--	--	--	--	--	0.5	2
M KNO_3	3	6	12	5	5	5	6	6	6	--	--	3	12	6	6
M $\text{Ca}(\text{NO}_3)_2$	2	4	8	--	2	8	4	4	4	4	5	5	4	4	4
M MgSO_4	1	2	4	2	2	2	--	1	4	2	2	2	2	2	2
0.05 M $\text{Ca}(\text{H}_2\text{PO}_4)_2$	--	--	--	--	--	--	--	--	--	10	10	10	--	--	--
M KH_2PO_4	--	--	--	1	1	1	1	1	1	--	--	--	--	--	--
0.5 M K_2SO_4	--	--	--	--	--	--	3	3	3	--	--	--	--	--	--
Supplement ^a	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1
5% FeCl	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1

^aSupplement supplied boron, manganese, zinc, copper, and molybdenum.

One quart plastic refrigerator containers coated with aluminum paint to exclude light from the rooting area were used to hold the solutions and plants. A large central hole in the container lid supported the seedling which was held in place by a circular wedge of polyurethane foam. A second hole at one corner of the lid was used to introduce additional iron solution into the medium through a piece of glass tubing. A third hole at the opposite corner of the lid was used for the aeration tube. The aeration device was a modification of that described by Harris (18). No filtering system was used and compressed air was provided by aquarium pumps.

During May to August the temperature and relative humidity in the greenhouse where the study was pursued varied from 22-35.5° C and 40-96 per cent, respectively. Eight seedlings per variety were transferred to each nutritional treatment. The respective nutrient solutions were added at a rate of 950 ml per container at 12-day intervals. Every 4 days 1 ml, 0.5 FeCl was added via the glass tubing to each container. When the volume of the solution fell below a third of its original level, it was refilled with distilled water.

Three plants per variety from each treatment were inoculated after they had been in their nutritional treatment for 55 days. The inoculum was applied directly to the upper surface of each leaflet from the 5th to the terminal leaf on the main stem only. An atomizer was used to apply the inoculum under a pressure of 5-6 lbs per square inch.

Inoculated plants were incubated for 3-4 days under the high relative humidity provided by enclosing all the foliage of each plant in a moist polyethylene bag. Leaflets similar to those inoculated were collected from the other plants and dried for 72 hours at 70° C.

On the 12th day after inoculation the percentage of inoculated leaflets with lesions was counted. After an additional 3 weeks a second disease rating was made and the percentage of defoliation was determined. On each occasion the actual number of lesions per leaflet was recorded. Conspicuous differences between lesion types were also observed.

The dried leaf materials were utilized in the following analyses. Total nitrogen was determined on the leaflets of 2 plants per treatment by Micro-Kjeldahl method (33). On the leaflets of 3 plants per treatment the following analyses were made: the atomic absorption method (6) was used to determine the calcium, magnesium, and iron content of the samples; potassium concentration was determined by flame emission spectrophotometry (41), and that of phosphorus colorimetrically (5). The leaflets from the 3 plants per treatment were ashed at 300° C for 1.5 hours and a further 4 hours at 550° C in a controlled electric muffle furnace. After the samples cooled in the oven, they were removed and 10 ml of 0.1 N HCl was added to each sample. After complete digestion, the solutions were filtered and brought to a volume of 50 ml with distilled water.

The experiment was repeated three times and mean data from the 3 experiments were subjected to analysis of variance. In several instances plants were lost and the missing mean values were calculated for use in the statistical analyses.

RESULTS

Deficiency Symptoms

Plants grown in calcium-deficient (Ca-0) nutrient solution did not live long enough to be utilized in the complete investigation. After 2.5 weeks, growth was already restricted, the apical portion of the plant was dead, and the older leaves had small brown necrotic spots. The first symptom expressed by plants grown in magnesium-deficient (Mg-0) nutrient solution after 3 weeks was interveinal chlorosis of the younger leaves. This was accompanied by stunted growth and reduction in the size of the leaflets. With advancing age, brown necrotic areas developed mainly along the midrib and chlorosis increased. Slight chlorosis or light green coloration of the youngest leaves was the first symptom due to potassium deficiency (K-0). At a later stage yellowing of the margins and necrotic "scorch" areas developed until the leaves were eventually shed.

There were no marked differences between the two varieties, 'Early Runner' and 'Florigiant', in their reactions to Ca-0, Mg-0, and K-0. However, in the case of phosphorus deficiency (P-0) the varieties reacted differently. In both varieties the symptoms were slow in appearing, the older leaves became yellow and eventually fell, and the terminal leaves were dark green and leathery. In the

'Early Runner' variety the main axis of each branch split along its length to the node where the last leaf was still intact, and the stem gradually dried up. The stems of 'Florigiant' variety remained normal except that they became reddish-brown. There were no discernible leaf symptoms on either variety in the other treatments, but overall growth differences, especially in plant size, were obvious.

Mineral Nutrient Levels and Disease

Levels of the elements calcium, potassium, magnesium, and phosphorus found in the respective mineral-treated plants are shown in Figures 1 and 2 and Tables 2 and 3. The latter, in addition, contain levels of iron and nitrogen. The levels of the 4 elements were significantly different in both varieties for all nutritional treatments except for potassium in 'Florigiant' (Table 4). The 14 nutritional treatments had a significant effect on the iron found in both varieties, but they had no such effect on the nitrogen contents of either variety. In all instances the increase of the respective nutrient in the mineral solutions was reflected as an increase in the level of that element in the plant's leaf tissue. Such a trend was not established in the complete solutions except in the case of the magnesium which increased with the increase in the concentration of the complete solutions. The levels of the elements in the leaves of both varieties increased and decreased most similarly in the magnesium and calcium treatments (Figures 1 and 2).

Figure 1. Mineral levels in leaves of 'Early Runner' variety grown in solutions containing varying concentrations of 4 elements.

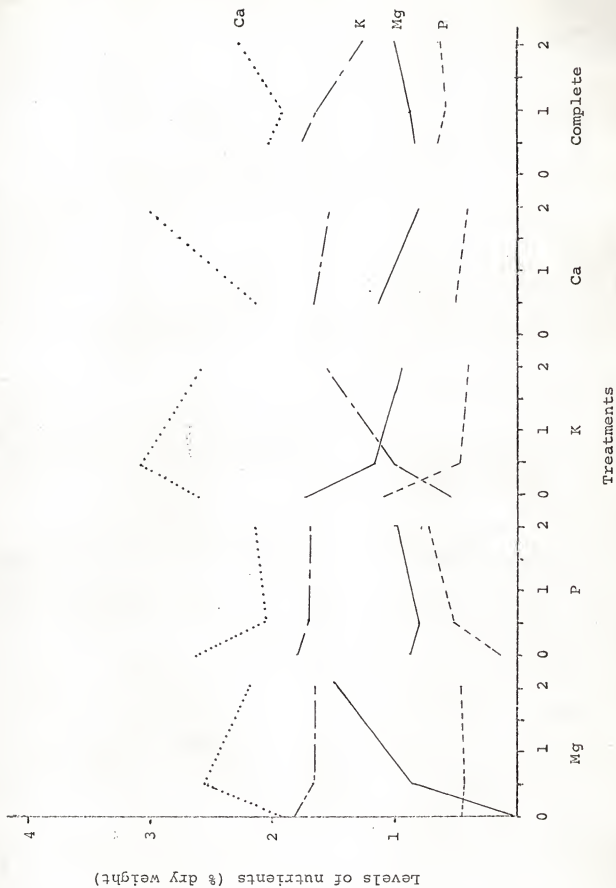


Figure 2. Mineral levels in leaves of 'Florigiant' variety grown in solutions containing varying concentrations of 4 elements.

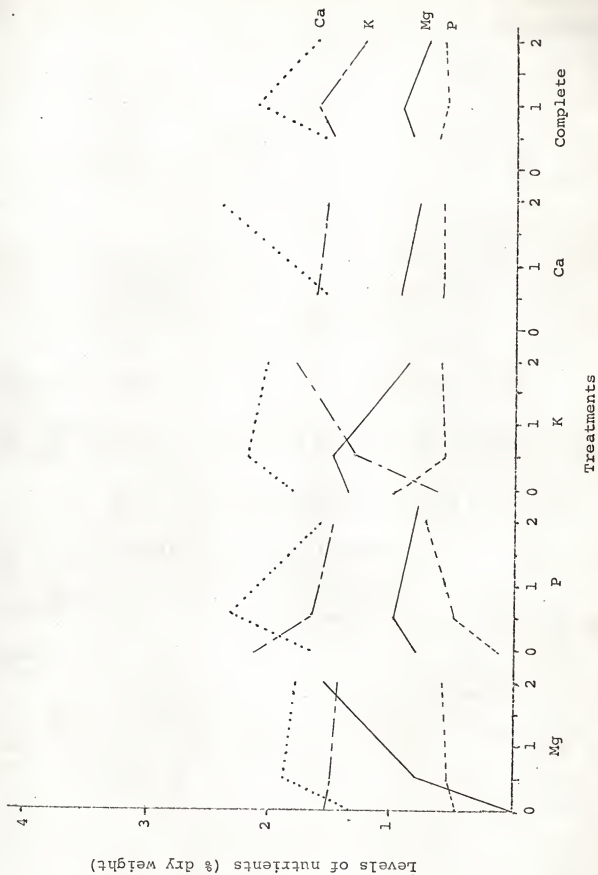


Table 2. Levels of nutrients in peanut leaf tissue of 'Early Runner' variety after growth in various nutrient solutions for 55 days.

Nutrient treatments	Mean ^a percentage/dry weight					ppm/ dry weight Fe
	Mg	P	K	Ca	N	
Mg-0	0.07	0.45	1.84	1.89	4.11	60
Mg-0.5	0.85	0.44	1.70	2.57	5.19	88
Mg-2.0	1.48	0.46	1.66	2.19	5.17	96
P-0	0.88	0.14	1.81	2.63	4.21	148
P-0.5	0.81	0.53	1.73	2.07	5.81	136
P-2.0	1.00	0.74	1.72	2.16	6.04	145
K-0	1.72	1.10	0.56	2.64	5.41	102
K-0.5	1.00	0.49	1.16	3.10	5.18	81
K-2.0	0.96	0.41	1.54	2.62	5.45	93
Ca-0.5	1.15	0.52	1.67	2.13	5.21	105
Ca-2.0	0.92	0.42	1.55	3.02	5.32	91
Complete-0.5	0.85	0.68	1.75	2.06	5.90	155
Complete	0.87	0.60	1.69	1.93	5.94	133
Complete-2.0	0.99	0.65	1.50	2.27	5.53	102

^a Values are means from 3 experiments.

Table 3. Levels of nutrients in peanut leaf tissue of 'Florigiant' variety after growth in various nutrient solutions for 55 days.

Nutrient treatments	Mean ^a percentage/dry weight					ppm/ dry weight
	Mg	P	K	Ca	N	Fe
Mg-0	0.05	0.47	1.55	1.36	3.35	61
Mg-0.5	0.80	0.55	1.53	1.90	5.56	91
Mg-2.0	1.59	0.60	1.47	1.82	5.17	101
P-0	0.82	0.12	2.12	1.67	4.17	177
P-0.5	1.02	0.47	1.68	2.32	5.70	176
P-2.0	0.92	0.74	1.49	1.59	5.67	94
K-0	1.39	0.99	0.64	1.84	5.72	95
K-0.5	1.50	0.58	1.33	2.20	5.47	82
K-2.0	0.90	0.60	1.81	2.05	5.44	115
Ca-0.5	0.96	0.61	1.66	1.60	5.74	110
Ca-2.0	0.81	0.62	1.58	2.42	5.49	165
Complete-0.5	0.87	0.65	1.52	1.58	5.83	178
Complete	0.93	0.57	1.64	2.14	5.70	147
Complete-2.0	0.71	0.60	1.26	1.64	5.88	111

^a Values are means from 3 experiments.

Table 4. Summary of analyses of variance for nutrient levels in leaves of two peanut varieties grown in various nutrient solutions.

'Early Runner'			'Florigiant'		
Source of variation	D.F.	Mean Square	Source of variation	D.F.	Mean Square
Magnesium					
Treatments	13	0.33 **	Treatments	13	0.36 **
Error	27	0.01	Error	25	0.04
Phosphorus					
Treatments	13	0.14 **	Treatments	13	0.10 **
Error	27	0.02	Error	25	0.02
Potassium					
Treatments	13	0.33 **	Treatments	13	0.25
Error	27	0.13	Error	25	0.12
Calcium					
Treatments	13	1.23 **	Treatments	13	2.56 **
Error	27	0.07	Error	25	0.73
Nitrogen ^a					
Treatments	13	0.86	Treatments	--	----
Error	24	14.6	Error	--	----
Iron					
Treatments	13	2467.6 **	Treatments	13	6041 **
Error	27	91.7	Error	25	1257

** Significance at 1% level

* Significance at 5% level

^a Mean from 3 experiments was not available for analysis in 'Florigiant'.

Disease ratings were based on percentage of leaflets with typical lesion symptoms, and the percentages obtained 33 days after inoculation were subjected to analysis of variance. Differences between nutrient treatments were statistically significant for 'Early Runner' but not for 'Florigiant' variety (Table 5). Plants of the 'Early Runner' variety grown in P-0 nutrient solution were most susceptible to infection by C. arachidicola; however, they were not significantly more susceptible than those grown in Ca-0.5, K-0.5, Mg-2.0, K-0, and Mg-0. Disease incidence of P-0 treated plants was significantly different from complete-2.0, K-2.0, complete-0.5, P-2.0, P-0.5, complete, Ca-2.0, and Mg-0.5. Plants grown in Mg-0 nutrient solution had the next highest disease incidence and were significantly different at the 5 per cent level from P-2.0, P-0.5, complete, Ca-2.0, and Mg-0.5 treated plants.

Table 5. Differences in susceptibility to Cercospora arachidicola of two peanut varieties grown in various nutrient solutions.

Solutions	% leaflets with lesions ^a 'Early Runner'	Solutions	% leaflets with lesions ^a 'Florigiant'
P-0	98 a	K-2.0	81
Mg-0	94 ab	P-0.5	79
K-0	79 abc	Mg-0	76
Mg-2.0	78 abc	K-0.5	74
K-0.5	74 abc	P-2.0	73
ca-0.5	71 abc	Complete-2.0	73
Complete-2.0	69 bc	Ca-0.5	71
K-2.0	68 bc	Mg-2.0	71
Complete-0.5	67 bc	Ca-2.0	69
P-2.0	65 c	Complete	68
P-0.5	65 c	Complete-0.5	68
Complete	59 c	Mg-0.5	58
Ca-2.0	59 c	P-0	56
Mg-0.5	54 c	K-0	48

^a Values are means of three replicates per experiment from 3 experiments; means followed by the same letter are not significantly different at the 5% level as measured by Duncan's multiple range test.

DISCUSSION

It is essential to procure a medium suitable for sporulation of C. arachidicola when large amounts of viable conidia are required for pathogenicity studies. Although C. arachidicola is a slow-growing fungus on most media, adequate quantities of conidia were obtained when I grew it on peanut leaf agar medium. Apparently, incorporating the host's tissue into the medium produces an adequate substrate for growth and sporulation. This has been shown to be the case not only with C. arachidicola and C. personata (1), but also for C. apii (27), C. beticola (39), and C. nicotianae (12). Abdou (1) concluded that peanut leaf extract contained the essential nutrient, probably thiamine, for spore initiation and formation in C. arachidicola and C. personata when added in the proper percentage. Refrigerating the medium for over 6 months did not interfere with its qualities as a satisfactory substrate in my study.

The few reports describing mineral nutrient deficiencies in peanut vary somewhat in their description of symptoms, but such inconsistency could be due to different investigational procedures and perhaps varieties. The different response of two varieties to phosphorus deficiency was clearly demonstrated in this work, but both varieties shared the

other symptoms typical of phosphorus deficiency and also those of calcium, magnesium, and potassium deficiencies in peanut (3, 7, 43).

Increasing each element separately in the nutrient solutions produced an increase of that element in the plant as indicated by the results of leaf analyses. Page (40) obtained similar results when potassium and calcium were applied as fertilizers to peanut fields. The increases in the nutrient solutions of the respective element were comparable to the concentrations of that element in the complete solutions. However, there was not an increase of the element in the leaf tissue as the element increased in the complete treatments except in the case of magnesium in 'Early Runner' variety. Such results indicate that in the complete solutions there is an interaction among the ions which interfered with their uptake by the plants.

Plants grown in magnesium and calcium solutions showed the least variation between varieties in the trend of accumulation of the elements in the leaf tissue although the levels of the accumulated elements differed. Calcium content in both varieties was decreased by an increase of either magnesium or potassium. Similar results were obtained by Eastman (13) and Page (40) with 'Virginia Bunch' variety in the field. Eastman's work also supported my findings that magnesium had little effect on the potassium content but potassium lowered the magnesium content. In contrast,

Burkhart and Collins (7) state that the lack of magnesium in the substrate had a greater effect on potassium accumulation in the leaves than the lack of potassium had on the magnesium accumulation. In addition they reported that potassium content was so great that it suppressed the uptake of calcium.

In this study plants grown in Mg-0 solution did not produce the most susceptible hosts as reported by Bledsoe et al. (2); instead the treatment that had the greatest predisposing effect on C. arachidicola infection was P-0 in 'Early Runner' variety and K-2.0 in 'Florigiant' variety. The disease incidence on Mg-0 plants was the second highest in 'Early Runner' and the third highest in 'Florigiant'. Although Shanta (46) did not indicate the variety used, she found that phosphorus and nitrogen fertilization decreased significantly disease incidence and potassium fertilization decreased it but the decrease was not significant. With respect to potassium similar results were obtained in my investigations with 'Early Runner' variety, but the reverse results were obtained with 'Florigiant'. Working with Chrysanthemum morifolium (Ramat.) Hemsl. and Botrytis cinerea Pers. ex Fr., Hobbs and Waters (23) found that the K₂O levels did not affect disease severity significantly; however, there was a trend toward fewer lesions as K₂O rates were increased. Reports of similar findings have been made by other workers (4, 10, 14) in their investigations with other disease systems.

Stall et al. (47) found that the percentage of phosphorus in tomato leaf tissue significantly increased, but the percentage of calcium did not when three rates of superphosphate were added to the soil, and under these conditions the amount of Botrytis gray mold significantly increased. In the variety 'Early Runner' a similar response to an increase of phosphorus was not indicated; instead the highest disease incidence occurred when phosphorus was deficient. In addition, Stall et al. (47) found that the least amount of disease always occurred where leaf calcium was high and leaf phosphorus was low. In my study the least amount of disease was found associated with the treatment Mg-0.5 which produced a low phosphorus level and a high calcium level in the leaves.

Sturgeon (48) found that the highest disease ratings were not associated with the greatest defoliation although infection was shown to be directly related to defoliation. My results show that the greatest amount of defoliation was associated with the highest disease rating in the 'Early Runner' variety but not in the 'Florissant' variety. The contradiction between my results and Sturgeon's may not be valid because the treatment, P-0, enhances premature defoliation. Therefore the possible relationship indicated in my study between greatest defoliation and highest disease may have resulted from the additive effects of nutritional treatment and infection on defoliation.

From the results obtained in this investigation, in addition to those in the literature reviewed, it is strongly suggestive that varieties of peanuts vary in their response to mineral nutrient treatments. Muse and Couch (38) in their studies with turfgrasses presented the concepts that (1) the mineral composition of a particular plant species is not fixed but may vary considerably with the cultural and climatic conditions under which the plants are grown, and (2) varieties of the same species may have distinctly different mineral compositions under identical environmental and nutritional conditions. The difference in disease susceptibility between the two varieties of peanuts when subjected to identical predisposing nutritional treatments and similar environmental conditions is supported by the latter concept. Furthermore, the variance with other investigators' findings on the effect of nutrition on susceptibility of peanut to C. arachidicola may be possibly explained by the concepts established by Muse and Couch (38).

SUMMARY

Peanut leaf agar medium was a satisfactory substrate for producing adequate quantities of conidia of Cercospora arachidicola Hori.

Typical deficiency symptoms for the elements calcium, potassium, magnesium, and phosphorus were produced in both varieties and no varietal differences existed except with phosphorus deficiency.

All 14 nutritional treatments produced significant differences in the levels of the elements under investigation in both varieties except for the potassium levels in the variety 'Florigiant'. In both varieties the concentrations of iron were also statistically different but not those of nitrogen.

The increase of a respective nutrient in the mineral solutions was reflected as an increase in the level of the element in leaf tissue except in the leaf tissue of the plants grown in complete solutions.

The varieties varied in their disease susceptibility response to the various nutritional treatments; significant differences occurred in the 'Early Runner' variety but not in 'Florigiant' variety. The highest and lowest disease incidence in 'Early Runner' variety was in phosphorus-deficient and magnesium-0.5 treated plants, respectively.

In 'Florigiant' variety, the highest and the lowest disease incidence was in magnesium-2.0 and potassium-deficient solutions, respectively.

No direct relationship between disease percentage and percentage of defoliation was indicated.

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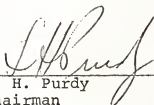
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BIOGRAPHICAL SKETCH

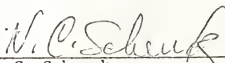
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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



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This dissertation was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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